

**Conclusion:** Community acquired meningitis by an organism of enteric flora in an immunocompromised patient should raise the suspicion of hyperinfection syndrome in endemic areas. Since eradication of parasite may be delayed, repeated stool examination and prolonged treatment are required.

## 118

### Unique Role(s) of Epstein Barr Virus Thymidine Kinase in Viral Pathogenesis

Xiaomin Zhao<sup>1</sup>, Mingxia Huang<sup>2</sup>, Joyce Fingerhuth<sup>1</sup>.

<sup>1</sup>BIDMC/Harvard Medical School, Division of Infectious Diseases, Boston, USA; <sup>2</sup>University of Colorado Health Sciences Center, Colorado, USA

**Background:** In patients with HIV/AIDS uncontrolled *Epstein Barr Virus* (EBV) replication produces high viral loads that expand the pool of latently infected cells vulnerable to development of B-lineage tumors: non-Hodgkins lymphoma (NHL) and classic Hodgkin lymphoma (HL). HAART accompanied by T-cell reconstitution dramatically reduced the incidence of EBV+ lymphomas, with the surprising exception of HL. Though EBV+ HL, can be distinguished from NHL by several criteria, a major difference is the grossly aneuploid nature of the malignant RS cell, a post-germinal center B-cell characterized by aberrant centrosomes and grossly abnormal chromosomes. Although latent cycle products alone were believed to promote viral tumorigenesis, accumulating evidence suggests lytic cycle proteins also play a critical role. The lytic enzyme EBV thymidine kinase (TK) is a modest dT kinase with a large N-terminus of unknown function. It is present in the virion tegument and interacts with multiple viral and cellular proteins forming a dominant node in the viral interactome. How and why these diverse interactions regulate virion production is entirely unknown. We recently found that unique among herpesvirus proteins, EBVTK strictly localizes to centrosomes. Significantly, upon lytic induction EBVTK is detected in centrosomes containing structural and numerical abnormalities. The centrosome is the master regulator of the microtubular cytoskeleton and controls the physical work of cell division. Minor perturbations of centrosome content cause errors in chromosome segregation, cytokinesis and cell cycle regulation, promoting aneuploidy and tumorigenesis.

**Objective:** To uncover the role(s) of EBVTK in control of lytic infection and in development of centrosome abnormalities.

**Methods:** Biochemical characterization of native enzyme. Construction and expression of wild type TK and a series of TK mutants followed by microscopy to detect localization and perturbation of centrosome/microtubule function in different cell types (yeast-human).

**Results:** EBVTK is a phosphoprotein in eukaryotic cells; phosphorylation plays distinct roles in different enzyme functions.

**Conclusions:** EBVTK is a multifunctional protein that performs distinct roles during different stages of the virus life cycle. TK expression may predispose to centrosome abnormalities resulting in aneuploidy as seen in EBV+ HL.

## 119

### Antifungal Activity of Posaconazole and Granulocyte Colony-Stimulating Factor in the Treatment of Disseminated Zygomycosis in a Neutropenic Murine Model

Stamatis Saoulidis<sup>1</sup>, Maria Simitsopoulou<sup>1</sup>, Maria Dalakiouridou<sup>1</sup>, Paraskevi Papaioannidou<sup>2</sup>, Emmanouel Roilides<sup>1</sup>. <sup>1</sup>3rd Department of Pediatrics, Hippokraton Hospital, <sup>2</sup>Dept. of Pharmacology, Aristotle University School of Medicine, Thessaloniki, Greece

**Background:** Zygomycosis is a life-threatening invasive fungal

infection most prevalent among immunocompromised patients. Posaconazole (POS) is a novel antifungal triazole with activity against zygomycetes. Granulocyte colony-stimulating factor (G-CSF) is known to augment neutrophil activity, in vitro, and improve the effects of antifungal drugs.

**Objective:** The aim of this study was to develop a neutropenic murine model of disseminated zygomycosis and evaluate the efficacy of POS alone or in combination with G-CSF.

**Methods:** Male Balb-c mice were infected via the lateral tail vein with  $5 \times 10^4$  conidia *R. microsporus*/mL. At 24 hour post-infection the mice were divided in four groups of five. Each group was treated with POS (40 mg/kg/day by gavage), or G-CSF (300 micro g/kg/day subcutaneously), or with the combination of POS+G-CSF. In the control group, animals were treated with saline. Mice were rendered neutropenic with cyclophosphamide (200 mg/kg, intraperitoneally) administered on days -1 and +5. Mortality was recorded for 10 days and survival was assessed with Kaplan-Meier curves. The fungal burden of the brain, liver, kidneys and lungs was evaluated with semi-quantitative cultures and statistical analysis was performed using arbitrary score units (n° mice).

**Results:** The mean survival times were  $6.56 \pm 0.57$  days for the controls,  $7.53 \pm 0.51$  days for POS-treated mice ( $P=0.098$  vs controls),  $6.93 \pm 0.56$  days for G-CSF-treated mice ( $P=NS$ ) and  $7.13 \pm 0.59$  days for POS+G-CSF-treated mice ( $P=NS$ ). Combination therapy ( $P=0.022$ ) as well as POS monotherapy ( $P=0.037$ ) reduced significantly the fungal burden in the kidneys. The reduction of fungal burden in the rest of the organs was not statistically significant.

**Conclusions:** Posaconazole monotherapy and POS+G-CSF combination therapy had a modest efficacy against *R. microsporus*. Furthermore, POS and combination therapy reduced significantly the fungal burden in the kidneys, while a moderate reduction of fungal burden was observed for the rest of the organs. Combining G-CSF with POS did not substantially affect the antifungal efficacy of POS.

## 120

### (1→3)-β-D-Glucan in Cerebrospinal Fluid is a Surrogate Marker for Detection and Therapeutic Response of Hematogenous Candida Meningoencephalitis

Ruta Petraitiene<sup>1,2</sup>, Vidmantas Petraitis<sup>1,2</sup>, William Hope<sup>1</sup>, Diana Mickiene<sup>1,2</sup>, Amy Kelaher<sup>1</sup>, Heidi Murray<sup>1</sup>, Christine Mya-San<sup>1</sup>, Johanna Hughes<sup>1</sup>, Margaret Cotton<sup>1</sup>, John Bacher<sup>3</sup>, Danny Bemjamin<sup>4</sup>, Thomas Walsh<sup>1</sup>.

<sup>1</sup>Immunocompromised Host Section, Pediatric Oncology Branch, National Cancer Institute, Bethesda, USA; <sup>2</sup>Laboratory Animal Sciences Program, SAIC-Frederick, Inc., Frederick, USA; <sup>3</sup>Surgery Service, Veterinary Resources Program, Office of Research Services, Bethesda, MD, Duke University, Durham, USA

**Background:** Hematogenous *Candida* meningoencephalitis (HCME) is a common complication of candidemia in immunocompromised pediatric patients, particularly neonates, resulting in abscesses, seizures, ventricular hemorrhage, mental retardation, and death. CSF cultures are often negative and early diagnosis of HCME is difficult.

**Objectives:** To characterize the potential diagnostic utility of CSF (1→3)-β-D-glucan as a biomarker for the detection and therapeutic monitoring of HCME.

**Methods:** The kinetics of expression of (1→3)-β-D-glucan in blood and CSF was studied in a well-established non-neutropenic rabbit model of HCME caused by *Candida albicans*. Levels of (1→3)-β-D-glucan were measured by factor G Limulus assay (Cape Cod Associates). Micafungin (MICA) (0.5 to 32 mg/kg/d IV) and deoxycholate amphotericin B (DAmB) (1 mg/kg/d IV) were administered to study the effects on expression of (1→3)-β-D-glucan for therapeutic monitoring.

**Results:** Among untreated controls (UC) (n=25), *C. albicans* es-

established multiples abscesses in cerebrum, cerebellum, choroid, vitreous humor, spinal cord, and meninges, where two (8.1%) of 25 CSF cultures were positive and 25 (100%) of simultaneous CSF samples for (1→3)- $\beta$ -D-glucan were positive with a range of 755 to 7750 pg/mL ( $p < 0.001$ ). Levels of (1,3)- $\beta$ -D-glucan in CSF were significantly greater than those of simultaneously obtained serum samples in treated animals ( $n=94$ ) and UC ( $p < 0.05$ ), suggesting CNS compartmentalization of this polymeric biomarker. Clearance of *C. albicans* from blood cultures was not predictive of eradication of organisms from CNS; whereas, reduction of CSF (1→3)- $\beta$ -D-glucan levels was predictive of therapeutic response. A significant decrease of (1→3)- $\beta$ -D-glucan concentration of CSF started at 0.5 mg/kg/day in comparison to that of UC ( $p < 0.001$ ). Fungemia persisted in all untreated controls. Positive blood cultures converted to negative with MICA and DAMB within one day after treatment, while CNS tissues remained positive for *C. albicans*. By comparison, CSF (1→3)- $\beta$ -D-glucan levels correlated directly in a dose-dependent pattern with therapeutic response and residual fungal burden of *C. albicans* in cerebral tissue ( $r=0.842$ ).

**Conclusions:** (1→3)- $\beta$ -D-Glucan in CSF is a surrogate marker for detection and therapeutic response of hematogenous candida meningoencephalitis.

## 121

### Species-Dependent Differences in Virulence of Medically Important Zygomycetes in Neutropenic Hosts are Related to Sporangiospore Germination, Hyphal Metabolism, and Circulating Molecular Biomarker Levels

Ruta Petraitienė<sup>1,4</sup>, Vidmantas Petraitis<sup>1,4</sup>, Charalampos Antachopoulos<sup>1</sup>, Johanna E. Hughes<sup>1</sup>, Margaret P. Cotton<sup>1</sup>, Susan M. Harrington<sup>2</sup>, Miki Kasai<sup>1</sup>, Andrea Francesconi<sup>1</sup>, Mara G. Beveridge<sup>1</sup>, Tin Sein<sup>1,4</sup>, Robert L. Schaufele<sup>1</sup>, John Bacher<sup>3</sup>, Dimitrios P. Kontoyiannis<sup>5</sup>, Thomas J. Walsh<sup>1</sup>. <sup>1</sup>National Cancer Institute, National Institute of Health, Bethesda, USA; <sup>2</sup>Clinical Cancer, National Institute of Health, Bethesda, USA; <sup>3</sup>Division of Veterinary Resources, National Institute of Health, Bethesda, USA; <sup>4</sup>SAIC-Frederick, USA; <sup>5</sup>MD Anderson Cancer Center, Houston, USA

**Background:** Zygomycetes are emerging fungal pathogens that cause life-threatening pneumonia in cancer patients, especially during prolonged neutropenia and corticosteroid therapy. Little is known about the relation between different species of Zygomycetes and their pathogenesis in pulmonary zygomycosis.

**Objectives:** To study the relative virulence of *Rhizopus oryzae* (RO), *Rhizopus microsporus* (RM), *Mucor circinelloides* (MC), *Mucor indicus* and *Cunninghamella bertholletiae* (CB) in experimental pulmonary zygomycosis and the possible correlation with germination rate, metabolic activity, and circulating zygomycete-specific DNA by qPCR.

**Methods:** Interspecies virulence was studied in experimental primary pulmonary zygomycosis in persistently neutropenic rabbits by a panel of validated outcome variables. Sporangiospore germination kinetics were measured over 4 h. Hyphal metabolic activity was determined by XTT assays. Plasma levels of zygomycete-specific DNA, as a surrogate biomarker for angioinvasion, were measured by qPCR of two regions within the 28S rRNA gene.

**Results:** There were significant inoculum-dependent differences in residual pulmonary fungal burden (CFU/g) among CB-, RM-, and RO-infected rabbits ( $10^2$ - $10^4$  CFU/g,  $p < 0.05$ ), and significant differences in organism-mediated pulmonary injury as measured by lung weights in RM-, and RO-infected rabbits ( $p < 0.05$ ). CB caused the highest lung weights, most extensive pulmonary infarcts, and lowest survival of 0% (0/18), in comparison to 16%

(3/18,  $p < 0.01$ ) of RM-, 81% (21/26) of RO- and 83% (15/18) of M-infections ( $p < 0.001$ ). Differences in virulence correlated with different germination kinetics at 4 h: CB (67-85%) > RM (14-56%) > RO (4-30%) > MC and MI (0%). These data correlated with greater in vitro metabolic activity by XTT assay of CB at 6 h (OD450=1.22) in comparison to that of RM, RO, MC and MI (0.37-0.84). Mean peak plasma zygomycete-specific DNA concentration (log GE/ml) followed a similar pattern: CB > RM > RO > MC.

**Conclusions:** Medically important species of Zygomycetes differ significantly in the outcome of pulmonary zygomycosis. *Cunninghamella bertholletiae* and *Rhizopus microsporus* were significantly more virulent than *Rhizopus oryzae* and *Mucor* species. Virulence parameters of zygomycosis in vivo correlate with species-dependent differences in germination kinetics, hyphal metabolic activity, and circulating levels of zygomycete-specific DNA.

## 122

### Characteristics of Transplant Recipients who Developed Influenza in 2007-08 despite Influenza Vaccination

Sherif Mossad. Department of Infectious Diseases, Cleveland Clinic, Ohio, Cleveland, USA

**Background:** Influenza vaccination is less immunogenic in transplant recipients than healthy people. Influenza A (H1N1) and B circulating viruses during the 2007-2008 epidemic were different from those contained in this season's vaccine.

**Objective:** To describe clinical and immunological characteristics of 15 transplant recipients who developed influenza despite influenza vaccination (group A), and compare them to 6 transplant recipients who developed influenza in the absence of influenza vaccination (group B), 13 transplant recipients who did not develop influenza (group C), and 8 healthy people who developed influenza (group D.)

**Methods:** Case ascertainment through microbiology and electronic medical records.

**Results:** Types of transplant: liver 3, heart 7, kidney 3, lung 8, HSCT 8, kidney + pancreas 3, liver + kidney 1, liver + pancreas 1. 20 had influenza A, and 10 had influenza B (1 transplant recipient has both simultaneously.) Influenza occurred 1757 days (mean [range 0-3850]) after transplant in group A, compared to 801 days (range 20-3082) in group B. Influenza occurred 116 days (mean [range 83-151] after vaccination in group A. There were no statistically significant differences in the incidence of fever, cough, rhinorrhea, sore throat, malaise, shortness of breath, exposure to contacts with similar symptoms, or presence of infiltrates on CXR among groups A, B and D. Groups A and B were treated with oseltamivir significantly more frequently than Group D, [ $p=0.002$ ]. Patients in group A were hospitalized more frequently than group D [ $p=0.0002$ ]. There were no statistically significant differences in the incidence of pneumonia, ICU admission, mechanical ventilation, or death among groups A, B and D. There were no statistically significant differences between groups A, B and C in the incidence of IgG < 600 mg/dL, immune function assay < 200 ng/mL, rejection (in SOT recipients) or GVHD (in HSCT recipients) in the preceding 30 days. Patients in group A were significantly more likely to have concomitant infections than group D [ $p=0.049$ ], but not group B.

**Conclusions:** Influenza vaccination did not alter clinical presentation of influenza in transplant recipients, or impact the incidence of complications. Transplant recipients who developed influenza despite influenza vaccination were not more immunosuppressed than those who were vaccinated and did not develop influenza, and were more likely to have concomitant infections than healthy people.